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Two-way communication: Volatile emission and uptake occur through the same barriers

Volatile organic compounds (VOCs) are released from aboveground organs into the atmosphere and/or from roots into the soil, allowing plants to communicate and interact with their environment, including with each other. Plant–plant communication via VOCs includes intra- and inter-species signaling as well as within-plant self-signaling. In some recipient tissues, for example, VOC perception encompasses storing the modified VOC for direct defense against herbivores (Sugimoto et al., 2014) or increasing tolerance to abiotic stresses (Zhao et al., 2020). In addition, or alternatively, perceived VOCs may initiate signaling cascades that prime plant defenses (Brosset and Blande, 2022). In all cases, plant VOCs are first released into the environment before being taken up and perceived by the same or neighboring plants. While significant progress has been made over the last several years to elucidate how VOCs are released from plant cells, these studies have also revealed new questions about how communicated VOCs are imported across cellular barriers. Recent reviews (Loreto and D’Auria, 2022; Wang and Erb, 2022) and a special issue (Vlot and Rosenkranz, 2022) have proposed mechanisms for VOC perception and signaling. In this Opinion piece, we shed light on the gaps in knowledge about VOC import and offer perspectives for future research aiming at understanding the molecular mechanisms underlying VOC-mediated communication in plants.

Plant VOCs directly vaporize from damaged cells and trichomes into the environment; however, those emitted from intact tissues must cross subcellular and plasma membranes, the aqueous cell wall, and either the cuticle or air spaces leading to stomata before reaching the environment. VOCs were long assumed to passively diffuse across each cellular barrier. For this to be true, plants would need to accumulate toxic levels of VOCs in cellular membranes to drive observed emission rates (Widhalm et al., 2015). These modeling predictions have since been validated by biochemical and genetic experiments showing that, in petunia flowers, a transporter is needed to traffic VOCs across the plasma membrane (Adebesin et al., 2017) and non-specific lipid transfer proteins facilitate VOC movement across the hydrophilic cell wall (Liao et al., 2022). In non-vegetative aerial tissues, the cuticle serves as a sink/concentrator for VOCs to modulate emission and protect cells from accumulating VOCs to toxic levels (Liao et al., 2021). VOCs in vegetative tissues may also take the same initial path but are more likely to diffuse from the cell wall into air spaces for release via stomata (Niinemets et al., 2014).

In order for plants to perceive and respond to volatile cues from their environment (Loreto and D’Auria, 2022; Vlot and Rosenkranz, 2022; Wang and Erb, 2022), VOCs must reach, and perhaps be taken up by, plant cells. Understanding this trafficking process at the (sub)cellular level is one of the major gaps in VOC-mediated communication. If VOC uptake occurs as emission in reverse, then VOCs from the environment will first favorably partition into cuticles or enter air spaces inside the plant via stomata (Figure 1A). From these locations, VOCs must then partition into the aqueous cell wall layer and cross it to reach the plasma membrane. Like emission (Liao et al., 2022), the movement of exogenous VOCs through the cell wall may be facilitated by LTPs (Matsui, 2016). The movement of VOCs through the cuticle, air spaces, and the cell wall is not compound specific. At the plasma membrane, however, VOCs may be recognized by binding to dedicated receptors that perceive specific VOCs and initiate signal transduction pathways. VOCs could also be transported across the plasma membrane via specific transporters or indiscriminately partition directly into the plasma membrane or extracellular vesicle membranes and enter the plasma membrane via endocytosis. VOCs that partition into the cell membrane will laterally diffuse and accumulate throughout the plasma membrane but could be pumped into the cell via transporters like that used to expel VOCs from the plasma membrane into the cell wall during emission (Adebesin et al., 2017). VOCs within the cell might then be partitioned into subcellular membranes or bind to compound-specific receptors localized in the cytoplasm, endoplasmic reticulum, or nucleus.

We set up a simple mathematical model of the uptake of VOCs to assess (i) if they will accumulate to sufficient levels for binding to protein receptors and transporters, (ii) if a biological concentrating mechanism is needed to approach the concentration for binding, or (iii) if VOCs taken up from the atmosphere build up to such high levels that they could damage cellular membranes (Adebesin et al., 2017; Liao et al., 2021). We modeled three representative VOCs involved in plant–plant communication, nerolidol, α-pinene, and cis-3-hexene-1-ol (Figure 1B) (Sugimoto et al., 2014; Brosset and Blande, 2022), at one part per billion (ppb), a value in the range of reported atmospheric plant VOC levels (Rasmussen and Went, 1965), which in SI units are 4.5, 7.4, and 10 nM for nerolidol, α-pinene, and cis-3-hexene-1-ol, respectively. Without protein-assisted uptake, VOC levels will eventually come to equilibrium at each barrier interface if the atmospheric concentration remains constant and if there are no detoxification or sequestration mechanisms in the receiver plant. The levels of VOCs within each barrier are a function of the partition coefficients for a VOC, where the air–cuticle ($K_{a/c}$), cuticle–water ($K_{c/w}$), and water–octanol ($K_{w/o}$) coefficients (Schmid et al., 1992) correspond to the atmosphere–cuticle, cuticle-cell wall, and cell wall–plasma membrane interfaces, respectively. In the case of

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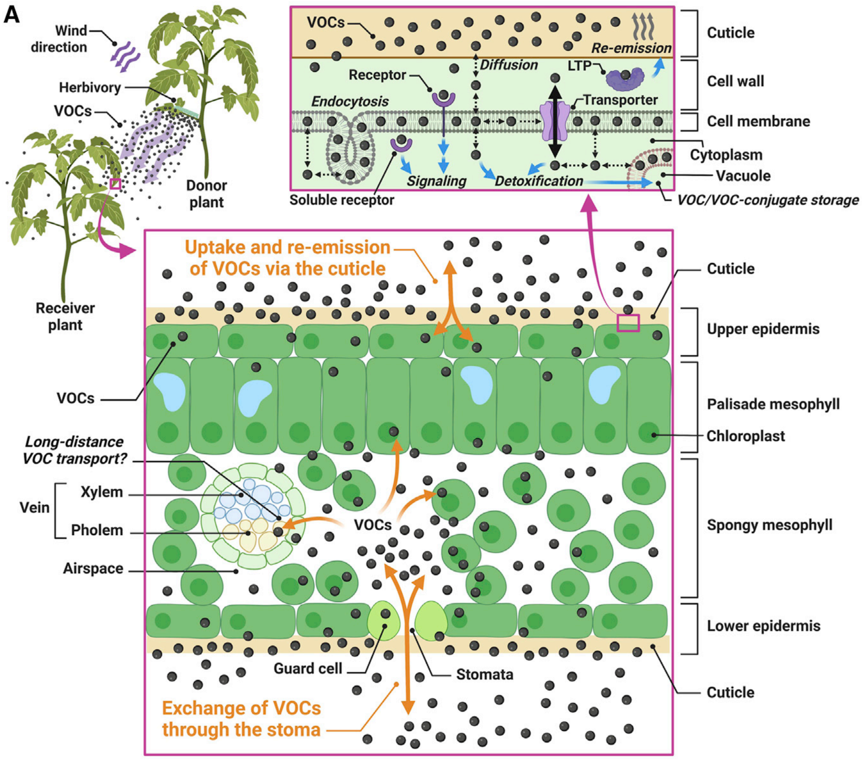

B

Cellular barrier/compound	Nerolidol	α-pinene	*cis*-3-hexene-1-ol
Cuticle	270 μM	68 μM	370 μM
Cell wall	3.6 nM	5.2 nM	15 μM
Plasma membrane	710 μM	160 μM	620 μM

Figure 1. Volatile organic compound (VOC) emission and uptake.

(A) Proposed bidirectional trafficking of VOCs through the same barriers in plant cells.

(B) Estimated distribution of plant VOCs in the cellular barriers at equilibrium. The estimated concentrations are based on partitioning of 1 ppb atmospheric concentration of nerolidol, α-pinene, and *cis*-3-hexene-1-ol. The partitioning between the atmosphere and aqueous cell wall is determined by the Henry's law constant for each compound, while the partitioning into the hydrophobic cell membrane is determined by the octanol-water partition coefficients (Schmid et al., 1992; United States Environmental Protection Agency Estimation Program Interface Suite. Ver. 4.11.), and calculations are extended to estimate the partitioning into the cuticle. The estimates neglect any effects of membrane pumps. Panel A was created with BioRender.com.

uptake via stomata, VOC levels between the atmosphere and cell wall are calculated using Henry's law ($K_{a/w}$). Our calculations revealed that at 1 ppb in the atmosphere, VOC concentrations in the cell will reach 68–370 μM in the cuticle, 3.6 nM–15 μM in the cell wall (independent of the path via cuticle or stomata) and cytoplasm, and 160–710 μM in the plasma membrane. Such concentrations are likely within the range of transporter K_ms and receptor K_ds, suggesting there is no need for a mechanism to concentrate VOCs in the cell prior to initiating signaling cascades. Internal VOC concentrations scale linearly with those in the atmosphere, thus if local VOC concentrations are higher in the immediate vicinity of a receiving plant, like upon herbivory on a neighboring plant (Niinemets et al., 2014), then it is possible that communicated VOC concentrations in receiver plant membranes may approach toxic levels. For example, if nerolidol reaches 10 ppb in the air, then the equilibrium concentration in the plasma membrane will be 7 mM. Detoxification of VOCs under equilibrium conditions would drive further uptake in plant cells (Matsui, 2016). In natural settings, however, equilibrium concentrations are likely not reached due to the transient release of communicated VOCs from donor plants and environmental factors like the wind that will disperse volatiles. Thus, as donor plant VOC concentrations go down, the rate of uptake into cells of receiver plant tissues will also decrease. It therefore seems unlikely that transient VOC intoxication occurs in receiver plants.

Under non-equilibrium conditions, the rates of VOC uptake will depend on whether they enter plant cells via stomatal air spaces or cuticles. In contrast to VOC emission, where stomata do not control the rate of release, entry through stomata likely plays a larger role in VOC uptake. When stomata are open, VOC concentrations in the atmosphere can equilibrate more rapidly with the air spaces inside plant tissues compared with the cuticle. This raises the question about the relative contribution of entry through the cuticle compared with stomata. In contrast to stomata, the cuticle provides a larger surface area for entry, which is likely important for capturing transiently communicated VOCs. Moreover, we hypothesize that VOCs partitioned into the cuticle will persist in plant tissues longer than those taken up via stomata as atmospheric concentrations drop. Captured VOCs may be re-emitted from cuticles (Niinemets et al., 2014), but it is also likely that these volatiles continue to diffuse into the cell long after the donor plant VOC signal was taken up and sustain VOC signaling in the receiver.

In addition to competing rates of VOC influx (diffusion plus protein-mediated trafficking, if any) and efflux (re-emission back out of the cell), the rates of VOC catabolism, modification, and/or sequestration (Figure 1) will determine the available level of VOCs for signaling (Niinemets et al., 2014). It is also possible that VOCs in their conjugated, modified, and/or catabolized forms function as signaling molecules. Metabolism of VOCs likely relies on the same detoxification processes used to eliminate other xenobiotics. If a VOC does not contain a reactive functional group, like a hydroxyl, then cytochromes P450 or other types of enzymes may first act on VOCs to introduce such groups. Next, a polar moiety, like sugar or glutathione, will be added by a transferase to increase the solubility of the compound and allow it to be more readily imported and stored and/or degraded elsewhere in the cell (Matsui, 2016). Enzymes controlling the detoxification of foreign compounds generally have broad substrate specificity and act on compounds belonging to different chemical classes as was shown for the glucosylation of VOCs by *Camellia sinensis* glucosyltransferases (Jing et al., 2019).

In summary, mathematical modeling of VOC trafficking into plant cells suggests that uptake through the cuticle or via air spaces, through the cell wall, and across the plasma membrane into the cell can occur solely by diffusion to reach nM to low μM concentrations, which are consistent with the expected range for binding to receptors or transporters or for further metabolism. This indicates that there is likely no need for a concentrating mechanism and that VOCs will not accumulate to toxic levels. How the molecular players involved in VOC uptake and signaling are regulated, and whether there is any type of developmental, temporal, or spatial coordination between the proteins, remains unclear. Development and testing of dynamic models for VOC emission and uptake are needed to more precisely pinpoint which barrier(s) provides the highest resistance. It remains an open question what the relative contribution of the cuticle and stomata to VOC uptake is. Furthermore, it should be experimentally investigated how the type of species and VOC(s), stomatal density, and environmental conditions contribute to uptake rate.

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Joshua R. Widhalm[1,2], *Meng-Ling Shih*[2,3], *John A. Morgan*[2,3] *and Natalia Dudareva*[1,2,4,*]

[1]Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907, USA
[2]Center for Plant Biology, Purdue University, West Lafayette, IN 47907, USA
[3]Davidson School of Chemical Engineering, Purdue University, West Lafayette, IN 47907, USA
[4]Department of Biochemistry, Purdue University, West Lafayette, IN 47907, USA
***Correspondence: Natalia Dudareva (dudareva@purdue.edu)**
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